United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Water Sampling Protocol
Revised April 2007

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Phytophthora ramorum is an oomycete, belonging to the group that includes *Pythium* species. Collectively these organisms are called "water molds" and are taxonomically related closer to algae than to fungi. For this reason, water collected from potentially infested nursery blocks must be tested for the presence of *P. ramorum*.

There are two potential methods provided here to detect *Phytophthora* species in water. The first uses rhododendron leaf baits in mesh bags followed by moist chamber incubation of the leaf baits. As of April 2007, research supports using leaves at least one year old, so that is recommended. Any suspect lesions that develop on the rhododendron leaves would be plated on PARP at 18-20°C (64-68°F). Any *Phytophthora* species growing on the PARP would need to be transferred to Corn meal agar or V8 agar for identification to species.

The second method uses water filtration. Water is removed from the pond, filtered with sterile filters and the filters placed on PARP. Once the filter is removed from PARP, any resultant *Phytophthora* colonies are transferred to Corn Meal Agar or V8 agar and identified to species.

***In situ* Water Sampling with Rhododendron Leaf Baits:**

A control sample using a leaf bait in distilled water should be run simultaneously with the leaf bait sample in the nursery site water. The USDA Forest Service recommends the following bait selection criteria in *Stream Baiting Protocol: 2007 National Phytophthora ramorum Early Detection Survey of Forests*, issued March 20, 2007. See http://fhm.fs.fed.us/sp/sod/sod.shtm for latest approved protocol.

Bait Selection

- Use leaves from a population of native or naturalized rhododendrons, if possible. The population should be sufficiently large to supply needed leaves for the survey duration.

- Variation in Pr susceptibility among rhododendron species/cultivars in laboratory inoculation has been published, but field and lab studies have

shown that leaves of common native and naturalized species perform acceptably as Pr bait.

- Leaf size can vary considerably among species and cultivars. If bait leaves are quite small (8 cm x 3 cm at the widest point or smaller), use 2 leaves in each pocket of the bait bag.

- If the source of leaves is nursery-grown or naturalized landscape plants, ensure that they have been free of fungicides and other pesticides for a minimum of 6 weeks before using as bait.
- Source plants should be mostly free of dieback and leaf symptoms. Use 1 year-old leaves as free as possible from leaf symptoms (spots, blight, and chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full leaf expansion and a period of hardening in summer.

- Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for stored leaves.

Prepare the rhododendron leaves as bait by trimming off the petiole end of each leaf. Place 3-4 cut leaves into a mesh bag. Label the bag with a plastic tag listing the date, water source (location), and nursery (i.e., nursery license number). Place the mesh bag into the water source for a minimum of 48-hours to 1-week (preferable). Do not leave the bait in the water source for longer than 1-week as the bait will begin to decompose. Place the bags such that the leaves will remain submerged the entire time (i.e., even if water levels fluctuate within the water source). If possible, place the bait near the influent coming from the area closest to or containing the infested plants.

Remove the bait from the water source and transfer to a sealable bag for transport to the laboratory. Label the bag with the information on the plastic tag, including the date collected. Log the leaf samples into the appropriate database. Assign a unique sample number to the bait(s) from each nursery.

Water Sampling for Filtration:

Water samples should be collected in a sterile wide-mouth bottle and kept at 5 – 10 C. Water samples should be taken from the surface to increase the likelihood of obtaining zoospores of *Phytophthora.*

Sample size should be approximately 1000 ml. Samples should be processed within 48 hours of collection or the samples should be discarded and new samples obtained and processed within 48 hours. Number of samples is determined by the size of the nursery pond to be sampled (Table 1)

Table 1: Number of composite samples collected based on pond size.

Size of pond (acres)	No. of water samples collected (liters)
0.00 - 0.25	5
0.26 - 0.5	10
0.50 - 1.0	20
>1.00	30

Note, if you have not used water filtration before and choose to do so, it is recommended you contact Dr. Steve Jeffers at Clemson University for further details on this technique.

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